ABSTRACT

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Differential scanning fluorimetry (DSF) is simple, rapid method that enables to determine optimal conditions for stabilization of proteins and discover their ligands. DSF monitors thermal unfolding of a protein in the presence of fluorescent dye (e.g. SYPRO Orange, 2,6-ANS). The dye is highly fluorescent in non-polar environment such as hydrophobic sites of unfolded protein that appear on the surface in gradual increase of temperature. Melting temperature (T_m) of a protein expresses its stability. Ligand screening relies upon the fact that protein stability is enhanced upon ligand binding ($\Delta T_m > 0$). The aim of this study was to introduce the DSF method on our department, use it for characterization of carbonyl reductase 1 (CBR1) and evaluate the obtained results with an independent methods and literature.

First, the functionality of method was verified with citrate synthase and the conditions were optimized for CBR1. The group of several buffers pH 3-10 without additives or with NaCl or glycerol were tested to choose an optimal environment for ligand screening of CBR1. Further, the influence of potential substrates and inhibitors of CBR1 on T_m were measured and correlated with independent method. As the optimal buffer for CBR1 potassium phosphate buffer, pH 7.4 was determined. DSF method proved that some substances from a group of potential substrates and inhibitors stabilize CBR1 ($\Delta T_m > 0$) so they are good ligands of CBR1. These results were in almost all cases correlated with independent methods for measuring an activity of CBR1. DSF method seems to be important tool in the era of genomic revolution for decrypting of function of uncharacterized proteins and will be widely utilized on Department of Biochemical Sciences.